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Multiple Lymphoid Nodules in Bone Marrow Have the Same Clonality as Underlying Myelodysplastic Syndrome Recognized With Fluorescent In Situ Hybridization Technique

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Benign nodular lymphoid lesions are not rare in the bone marrow of patients with myelodysplastic syndrome (MDS). Herein, we report a case of MDS with clonal lymphoid aggregates in the bone marrow but without evidence of systemic lymphoma. The case of a 71-year-old man was evaluated for cytopenia. His bone marrow was initially hypocellular, with 10% blasts and a few small lymphoid aggregates. The diagnosis of refractory anemia with excess blasts was made. The disease progressed gradually, and he received erythropoietin and granulocyte colony-stimulating factor for a short time. Forty-two months later, acute leukemia (M1) developed, with 60% to 70% blasts in the bone marrow. The bone marrow also showed large aggregates of lymphocytes. Immunohistochemical study of these cells in the nodular lesions showed 50% CD3+ and 50% CD20+. Cytogenetic and molecular genetic studies revealed monosomy 7 and T- and B-cell clonal gene rearrangement. Fluorescent in situ hybridization study with centromere-specific probes of a bone marrow specimen showed monosomy 7 in both nodular lymphoid lesions and surrounding bone marrow cells, indicating that both processes originated from the same abnormal pluripotential progenitor. Am. J. Hematol. 59:252–257, 1998.

Key words: myelodysplastic syndrome (MDS); in situ hybridization; lymphoid nodule

INTRODUCTION

Benign reactive lymphoid aggregates are not uncommon in bone marrow samples and are found with increasing frequency with advanced age [1]. These nodules have no specific pathologic significance but need to be differentiated from low-grade lymphoproliferative disorders, which also are more common in the elderly than in the young.

Lymphoid aggregates can be found in the bone marrow of patients with myelodysplastic syndrome (MDS). In a review by Rios et al. [2], these aggregates were present in 10% of 120 cases analyzed. The coincidental association of low-grade lymphoproliferative disease in MDS is a rare event (estimated at 1%) [3]. In addition, molecular genetic evidence of polyclonal or monoclonal lymphoid expansion in MDS has been described in 7% to 10% of patients. In cases with monoclonal expansion, gene rearrangement suggests that these cells arise from the same pluripotential precursor that causes the myeloid dysplastic process [4].

Herein, we describe the case of a 71-year-old man with refractory anemia and excess blasts that evolved to acute leukemia over a period of four years. Throughout the course of his illness, we documented a clonal T- and B-cell expansion that proved to arise from the same clone of abnormal pluripotential precursor cells as the acute leukemia.

Case Report

A 71-year-old retired radiologist had a history of peptic ulcer disease treated with partial gastrectomy and

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Billroth II anastomosis. He had experienced dyspepsia most of his adult life. In 1989, he was found to have gallstones. One year later, he became more symptomatic and ultrasonography showed a 2.5-cm polypoid mass in the gallbladder. At surgery, this lesion was discovered to be an invasive grade II adenocarcinoma that had metastasized to one of two regional lymph nodes.

After cholecystectomy, the patient received adjuvant chemotherapy (5-fluorouracil) and radiation therapy (5,040 cGy). One and a half years later, in July 1992, he was first noted to have mild anemia, with macrocytic indices, and leukopenia. He was given injections of vitamin B12, but a complete blood cell count three months later showed no improvement. A peripheral blood smear revealed many large granular lymphocytes. His bone marrow was hypocellular (10% cellularity), with megaloblastoid and dysplastic red blood cell precursors. Granulopoiesis was left-shifted, with 10% to 15% blasts; a few small lymphocytic aggregates were also noted. Of 20 metaphases, six had deletion of the Y chromosome, but no other cytogenetic abnormality was noted.

Because the patient was asymptomatic, it was elected to treat with observation. During the next two years (1992 to 1994), the pancytopenia progressed. A repeat bone marrow examination in March 1994 showed hypocellularity (20% cellularity), with increased blasts (15% to 20%) and nodular lymphoid aggregates involving 10% of the bone marrow. Flow cytometric analysis showed an increased proportion of myeloblasts expressing CD13, CD33, and CD34 and the presence of a small monoclonal kappa B-cell population. Both T- and B-cell gene rearrangement showed minor clones of each population.

During the subsequent two years, the patient received intermittent treatment with erythropoietin and granulocyte colony-stimulating factor. The hemoglobin value was stable at nine to 10 g/dL, and he required transfusions only infrequently.

In March 1996, the patient had progressive fatigue and other symptoms of anemia. A repeat bone marrow examination revealed hypercellularity (60% cellularity), with extensive myeloblast (myeloperoxidase-positive) infiltration (80%) and an increased number of nodular lymphoid aggregates. Cytogenetic analysis revealed monosomy 7 in 22 of 31 metaphases. The patient chose not to have aggressive ablative therapy; one month later he died at home.

METHODS

Immunohistochemistry for Determining the Cell Type

Sections were cut from bone marrow biopsy blocks fixed in B5 (mercury chloride/formaldehyde), mounted on slides, and deparaffinized in xylene and xylene/iodine solution after being dried in a 60°C oven for 60 min and hydrated in two concentrations of ethanol (absolute and 95%). Next, endogenous peroxidase activity was blocked with a 1:1 solution of 3% hydrogen peroxide and absolute methanol. After this, the slides were stained according to the labeled streptavidin-biotin immunoperoxidase method with the Ventana system (Tucson, AZ) at 37°C to 42°C, using the specific antibodies for CD3, CD20 (L26), CD68 (PG-M1) (DAKO, Glostrup, Denmark) (Table I).

TABLE I. Antibodies Used in the Study*

Antibody	CD	Dilution	Pretreatment	Specificity
Anti-CD3	CD3	1:50	Pepsin-microwave	T cells
L26	CD20	1:50	Steam-citrate	B cells
PG-M1	CD68	1:100	None	Monocytes

*Source for all antibodies was DAKO (Glostrup, Denmark).

Appropriate isotype-matched positive and negative controls were processed in parallel with each group of immunostained slides.

Southern Blot Analysis

As described previously [5], DNA was extracted from peripheral blood or bone marrow with the phenol/chloroform technique and digested to completion with restriction enzymes. Electrophoresis was performed on an agarose gel to separate the DNA fragments, which were then transferred to a nylon membrane. The membrane with *Eco*RI-digested DNA was hybridized with 32P-GTP-labeled constant region probe to the λ light chain, first (Jβ1) and second (Jβ2) joining regions of the β-chain and joining region of the γ-chain of the T-cell receptor. The *Bgl* membrane was hybridized to the joining region probe of the heavy chain (J_H). One of two *Bam*HI membranes was hybridized with a constant region probe of the heavy chain, whereas the other was hybridized to the joining region probe to the κ light chain. Autoradiography was used to detect both the germ line and the rearranged fragments within the heavy chain, light chain, and T-cell receptor gene.

In Situ Hybridization With Centromere-Specific Probe

Paraffin-embedded tissue was cut into 4-μm-thick sections, which were deparaffinized in three changes of xylene (Histo-Clear, National Diagnostics, Atlanta, GA), 8 min each, at room temperature. They were rehydrated in 100% ethanol (3 times, 5 min each) and boiled in a microwave oven with 10 μM citric acid at pH 6 for 10 min. Next, the sections were digested in pepsin solution (4 mg/mL in 0.9 NaCl at pH 1.5; Sigma Chemical Co., St. Louis, MO) for 15 min at 37°C, rinsed twice in standard saline citrate (300 mM sodium chloride and 30 mM

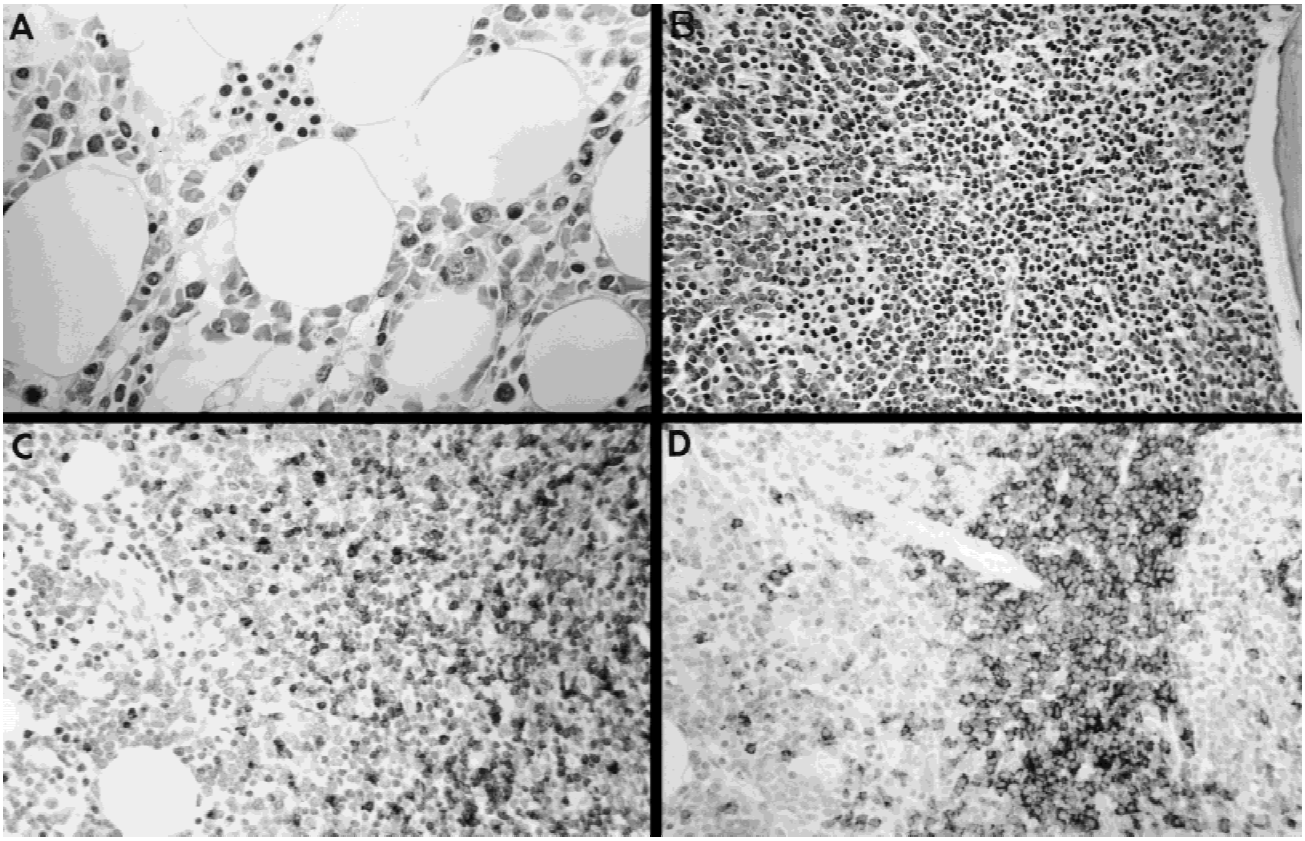

Fig. 1. A, Initial bone marrow specimen (10/92) from patient showing hypocellular marrow with relative increase of blasts consistent with refractory anemia with excess blasts. B, Subsequent bone marrow specimen (3/96) showing increased cellularity and blasts (larger cells in the left upper and far left of the field) and aggregate of small lymphocytes (small cells in the right side of the field). (A and B, Hematoxylin-eosin; original magnification, ×400.) C, Immunostaining of the same marrow specimen as in B, showing presence of approximately 50% of CD3+ T lymphocytes in lymphoid aggregate. (Immunoperoxidase stain with antibody for CD3; original magnification, ×400.) D, Immunostaining of the same marrow specimen as in C, showing presence of approximately 50% of CD20+ B lymphocytes concentrated at center of lymphoid aggregate. (Immunoperoxidase stain with antibody for CD20; original magnification, ×400.)

sodium citrate) at room temperature for five min/wash and air dried. Because cytogenetic studies showed abnormalities for chromosome Y and 7, direct-labeled fluorescent DNA probes (Vysis, Framingham, MA) for the centromere regions of chromosome Y and 7 were chosen for the study. The probes and target DNA were denatured simultaneously in an 80°C oven for five min, and each slide was incubated overnight at 37°C. After hybridization, the slides were washed twice in standard saline citrate for five min each at room temperature, in 1.5 M urea/0.1 × standard saline citrate at 45°C twice for 12 min each, and, again, twice in standard saline citrate for five min each at room temperature. Nuclei were counterstained with 4,6-diamidino-2-phenylindole and the antifade compound P-phenylenediamine.

More than 50% of nuclei with missing signals (0 or 1) was interpreted as chromosome deletion. For an addi-

TABLE II. Results of Southern Blot Analysis of Peripheral Blood and Bone Marrow*

		Date of study				
		7/93	3/94		3/96	
Genes	Region	PB	PB	BM	PB	BM
T-cell receptor						
β-Chain	J1 (*Eco*RI)	−	−	−	−	−
	J2 (*Eco*RI)	+	+	−	−	−
γ-Chain	J (*Eco*RI)	+	+	−	E	−
Immunoglobulin						
μ Heavy chain	C (*Bam*HI)	ND	−	−	ND	ND
	J (*Bgl*II)	ND	−	−	ND	ND
κ Light chain	J (*Bam*HI)	ND	−	+	ND	ND
λ Light chain	C (*Eco*RI)	ND	−	QNS	ND	ND

*BM, bone marrow; E, equivocal result; ND, not determined; PB, peripheral blood; QNS, quantity not sufficient; +, clonal gene rearrangement noted; −, no rearrangement noted.

TABLE III. Results of Regular Cytogenetic Studies and Fluorescent In Situ Hybridization (FISH) Studies*

			Date of study		
Bone marrow	Location	Normal value	10/92	3/94	3/96
Cellularity, %		30–50	10	20	60
% blasts		<5	10	20	70
Karyotype		46,XY	45,X,−Y[6]/46,XY[14]	46,XY[20]	45,XY,−7[22]/46,XY[9]
FISH	Nodular[a]		−Y[b]	−Y[b]	−7[b]
	Marrow[c]		N	N	−7[b]

*N, within normal limits.
[a]Nodular lymphoid aggregates.
[b]More than 50% of nuclei with missing signals.
[c]Bone marrow surrounding nodular aggregate.

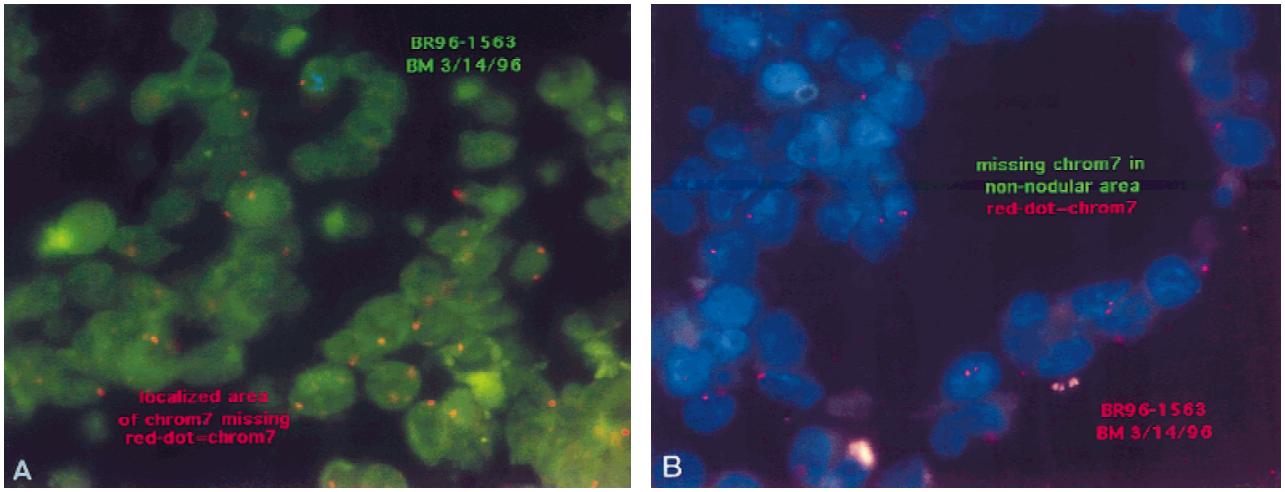

Fig. 2. Fluorescent in situ hybridization shows monosomy 7 in both nodular lesion (A) and surrounding marrow (B). Because of the thickness of the sections, only a fraction of the cells in the field are in focus and show clear signal in the cells. Most of the cells in focus showed one signal for chromosome 7 and only rare cells showed two signals.

tional chromosome, we used more than 8% of the cells with extra signal (⩾3).

RESULTS

Immunohistochemical Study

Most of the cells in the nodular lesions were monomorphic small lymphocytes with scant cytoplasm and round nuclei. About half of these cells were strongly positive for CD3 and the rest were positive for CD20 but negative for PG-M1, KP-1, κ light chain, and λ light chain (Fig. 1).

Southern Blot Analysis

Southern blot analysis of the peripheral blood showed clonal gene rearrangement in the second joining region of the β-chain (Jβ2) and joining region of the γ-chain of the T-cell receptor. Bone marrow also showed clonal gene rearrangement in the joining region of the κ light chain (Table II).

Fluorescent In Situ Hybridization With Centromere-Specific Probe

In March 1996, fluorescent in situ hybridization with a centromere-specific probe for chromosome Y and 7 demonstrated that the cells in both the nodular lesions and the surrounding bone marrow were monosomy 7 (Fig. 2). However, similar studies performed in October 1992 and March 1994 showed chromosome Y deletion in most of the cells in the nodular areas (Table III).

DISCUSSION

MDS generally is considered a clonal disorder that originates from pluripotent stem cells [6–9]. It can occur spontaneously or as a result of chemotherapy [10,11] or irradiation [12]. Oftentimes, the disease evolves to acute leukemia. Most often, it is a myeloid process, but acute lymphoblastic leukemia has also been reported [13,14]. The mechanism for this progression is not known.

We report a case of MDS that developed 18 months after the patient received treatment with 5-fluorouracil and irradiation for gallbladder carcinoma. Of interest, this patient had nodular lymphoid lesions in bone marrow tissue, which contained both T- and B-cell clonal populations (50% CD3+ and 50% CD20+). Cytogenetic studies showed 45,XO in some of the cells in the initial bone marrow specimen (October 1992) and monosomy 7 of a subsequent bone marrow specimen (March 1996). Southern blot analysis revealed both T- and B-clonal gene rearrangement. The patient had no evidence of systemic lymphoma. The abnormal B-cell clone appeared to be localized in the bone marrow. This clone may have been a nidus of evolving lymphoma in the future if the patient had not died of acute leukemia.

The coexistence of MDS with a lymphoid or plasmacytic neoplasm has been reported [15–18], but the incidence is low in comparison with the lymphoid aggregates found in about 8% of cases with myelodysplasia [2]. The results of clonal studies in MDS are still equivocal; some suggest that the pluripotent stem cell is affected in MDS and others suggest that the lymphoid progenitor is not involved because the lymphoid cells usually do not express the abnormal karyotype [19–23]. In our patient, clonal lymphoid nodules grew up during follow-up, and fluorescent in situ hybridization showed the same chromosomal abnormality (monosomy 7) in the nodular area and in the surrounding bone marrow, which supports that the disease arose from a pluripotential progenitor. Perhaps the loss of the Y chromosome should not be considered a marker of the malignant clone, because in one large study group the incidence of −Y in MDS was not different from that in normal controls (normal: MDS = 7.7%:10.7%) [24].

CONCLUSIONS

We report a patient with MDS who had clonal nodular lymphoid lesions (both B and T cells) in the bone marrow. These cells had the same clonal aneuploidy as the surrounding bone marrow, suggesting that they might be the same clone or subclone as the cells in the surrounding underlying myelodysplasia.

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